REMARKS/ARGUMENTS

Status of the Claims

Claims 12-19 were rejected. Claims 1-11 were previously cancelled without prejudice or disclaimer. Applicants reserve the right to pursue these claims in a continuation or divisional application. Claims 12-19 are pending in the present application.

The Objection to the Drawings Should Be Withdrawn

The Examiner has objected to the drawings for failure to comply with the requirements of 37 CFR 1.84. New formal drawings have been submitted. In light of the submission of new drawings, the objection has been obviated and should be withdrawn.

The Rejection of the Claims Under 35 U.S.C. § 103 Should Be Withdrawn

Claims 12-16 and 18 were rejected under 35 U.S.C. § 103 as being unpatentable over Woghiren *et al.* (1993) *Bioconj. Chem.* 4:314-318 in view of Miles *et al.* (1997) *Art. Cells, Blood Subs., Immob. Biotech.* 25:315-326, Iwashita *et al.* (1988) *Biomat., Art. Cells, Art. Org.* 16:271-80, Rausch *et al.* (U.S. Pat. No. 5,084,558), Katsunuma (U.S. Pat. No. 4,229,571), and JP 53038617. This rejection is respectfully traversed.

A *prima facie* case of obviousness requires some suggestion to combine the cited references to arrive at the claimed invention and a reasonable expectation of success in such a combination. The claimed invention in the instant case is a method of preparing a chemically modified hemoglobin that is substantially free of contaminants comprising dissolving an aPEG in a solvent in which the aPEG is stabile, filtering the aPEG solution to substantially reduce the level of contaminants, and combining the filtered aPEG solution with a hemoglobin solution. Significant reductions in contaminants present in the chemically modified hemoglobin solution result from using a filtered aPEG solution. See, for example, pages 15-17, Example 4 and Tables 2-3. The Examiner asserts that the motivation to combine the cited references to arrive at the claimed methods arises from the desirability of producing a safer, non-toxic hemoglobin solution for use as a therapeutic. This reasoning is insufficient to establish a motivation to combine the

references. Moreover, the references, even if combined, would not allow one of skill in the art to produce the claimed invention.

Woghiren *et al.* teach a thiol-protected activated PEG (aPEG) and a method of using it for the modification of cysteine-containing proteins. Specifically, monomethoxypolyethylene glycol (mPEG) is transformed to a thiol-protected aPEG (i.e., PEG-SS-4TP) through a series of synthetic steps, and the resulting PEG-SS-4TP is used to modify papain. Woghiren *et al.* do not teach or suggest chemically modifying hemoglobin.

The production of PEG-SS-4TP requires several intermediate reactions, such as the preparation of tosyl-PEG, the preparation of PEG-thioacetate, alcoholysis of PEG-thioacetate, and protection of the free thiol, in order to produce the final desired aPEG (i.e., PEG-SS-4TP) that is used to chemically modify papain. At each of these steps, the reaction products are separated from the residual chemical reagents by filtration through Sephadex G-25. The reference does not teach or suggest that this filtration step is intended to or actually does substantially reduce bioburden and endotoxin contaminants. In fact, Woghiren *et al.* expressly state that the reaction products are only "partially purified" by Sephadex G-25, indicating that the filtration step does not substantially reduce the level of contaminants, as required by the present invention. See, for example, page 315, right column. Furthermore, in the final synthetic step, the PEG-SS-4TP is isolated by gel filtration as before, frozen, and evaporated to dryness using a vacuum concentrator. The papain protein solution is then added drop-wise to a 15-fold molar excess of PEG-SS-4TP, resuspended in MOPS buffer, and the resulting PEG-papain solution is purified by HPLC and high-performance gel filtration chromatography. Thus, contrary to the claimed methods, the final aPEG solution that is used to modify papain is not filtered prior to use. Moreover, there is no suggestion in the reference that PEG-SS-4TP could be successfully used to modify hemoglobin.

In contrast to the teachings of Woghiren *et al.*, the present invention requires dissolving the aPEG in a solvent in which it is stable, filtering the solution to substantially reduce bioburden and endotoxin contaminants, and then using the filtered aPEG solution to modify hemoglobin. As discussed above, the filtration steps in the methods of Woghiren et al. do not substantially reduce contaminants, and, moreover, the reference simply does not teach or suggest filtering the

final PEG-SS-4TP solution prior to using it to modify papain. In fact, the modified papain solution is purified to substantially reduce contaminants only after the chemical modification has occurred. Furthermore, Woghiren *et al.* teach only the modification of papain and do not even suggest using the disclosed method to produce a chemically modified hemoglobin solution.

Katsunuma discloses compositions and methods directed to a glucocorticoid sparing factor (GSF). JP 53038617 teaches a method for preparing an inactivated hepatitis B vaccine. Both references generally teach the use of gel filtration and column chromatography for the purification and separation of compositions of interest. Neither reference, however, is directed to hemoglobin solutions or suggests filtering an aPEG solution.

Miles *et al.* teach an HPLC-based method for the quantitation of residual α-carboxymethyl, ω-carboxymethoxy polyoxyethylene (POE) in chemically modified hemoglobin solutions. The cited reference further acknowledges that the removal of residual chemical modification reagents (e.g., POE) is crucial to the production of a safe product. Iwashita *et al.* teach compositions and methods directed to a pyridoxalated hemoglobin-POE conjugate. The modified hemoglobin of Iwashita *et al.* is purified by repeated ultrafiltrations of the final product. Rausch *et al.* disclose a cross-linked, substantially endotoxin-free hemoglobin solution and a method for producing the same. The methods of Rausch *et al.* comprise several filtrations of the unmodified hemoglobin solution in order to remove endotoxin contaminants prior to cross-linking with glutaraldehyde. None of these references, however, teach or suggest using a stable, filtered aPEG solution to modify hemoglobin.

The Examiner asserts that in light of the recognized need for purified hemoglobin solutions one skilled in the art would have been motivated to combine the method of Woghiren *et al.* with the hemoglobin solutions of Iwashita *et al.* or Rausch *et al.* and the disclosure of general gel filtration techniques of Katsunuma to arrive at the claimed method. None of the cited references, however, teach or suggest dissolving an aPEG in a solvent in which it is stable and then filtering the aPEG solution to substantially reduce the level of contaminants prior to using it to modify hemoglobin, as required by the claimed invention. Therefore, there is insufficient motivation to combine the cited references to obtain the claimed invention, and, as such, a *prima facie* case of obviousness has not been established.

Furthermore, although there is insufficient motivation to combine the references, even if combined, the references would not allow one of skill in the art to arrive at the claimed invention. As discussed above, none of the references cited by the Examiner teaches dissolving an aPEG in a solvent, filtering the aPEG solution to substantially reduce bioburden and endotoxin contaminants, and combining the filtered aPEG solution with hemoglobin, critical steps in the claimed methods. Moreover, prior to the present disclosure it was not known that a stable aPEG solution could be produced, filtered to substantially reduce bioburden and endotoxin levels, and successfully used to modify a hemoglobin solution. Accordingly, the combination of cited references could not have placed the invention of claims 12-16 and 18 in the hands of the public, and a *prima facie* case of obviousness under 35 U.S.C. § 103 has not been established.

The Examiner further concludes that claim 17 and 19 are obvious in view of the above references as modified by Feola *et al.* (U.S. Pat. No. 5,439,882). Dependent claims 17 and 19 further comprise filtering the aPEG solution through a 0.2 micron nylon filter. Feola *et al.* teach the use of a 0.2 micron Posidyne® filter to remove contaminants from an extracted, unmodified hemoglobin solution. The Examiner maintains that it would have been obvious to one of skill in the art to use the filter disclosed by Feola *et al.* in conjunction with the method of Woghiren *et al.* and the disclosures of Miles *et al.*, Iwashita *et al.*, or Rausch *et al.* to arrive at the methods of claim 17 and 19. The Examiner further asserts that Miles *et al.* teach the use of a micron-sized filter to purify a POE solution, and, therefore, it would have been obvious to use the filter of Feola *et al.* to filter an aPEG solution.

First, the Examiner's attention is drawn to page 316, "Materials and Methods" of Miles *et al.*, which clearly indicates that it is the pyridoxalated hemoglobin-POE conjugate samples, and not the POE solutions, that were filtered using a 0.45 micron filter. Thus, contrary to the Examiner's assertions, Miles *et al.* does not suggest filtering POE or any aPEG solution using a filter of any size. Second, in contrast to claims 17 and 19, Feola *et al.* disclose only the use of a 0.2 micron filter to remove contaminants from an extracted hemoglobin solution. Feola *et al.* do not even teach or suggest using an aPEG to chemically modify a hemoglobin solution, and, therefore, also do not suggest using any filter to remove contaminants from an aPEG solution. The mere fact that Feola *et al.* use a 0.2 micron filter to remove contaminants from a hemoglobin

solution is no indication that one of skill in the art would have been motivated to dissolve an aPEG in a solvent in which it is stable, filter the aPEG solution through a filter of any type to substantially reduce contaminants, and then use the filtered aPEG solution to modify hemoglobin. Moreover, as noted above, none of the cited references teach or suggest filtering an aPEG solution to substantially reduce bioburden and endotoxin levels prior to using the filtered aPEG solution to chemically modify hemoglobin. Therefore, claims 17 and 19 are not obvious in view of the cited references.

For the reasons presented above, the Examiner has failed to establish a *prima facie* case of obviousness. Accordingly, Applicants respectfully submit that the claimed methods for producing a chemically modified solution substantially free of contaminants are not obvious in view of the cited references and request that the rejection of claims 12-19 under 35 U.S.C. § 103(a) be withdrawn.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

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